Mutants-P318A.ST25
SEQUENCE LISTING

<110> Covalys Biosciences AG

<120> Mutants of O6-Alkylguanine-DNA Alkyltransferase

<130> P318A

<150> EP04405123.3
<151> 2004-03-02

<150> EP04405465.8
<151> 2004-07-22

<160> 48

<170> PatentIn version 3.3

<210> 1
<211> 624
<212> DNA
<213> Homo sapiens

<400> 1
atggacaagg attgtgaaat gaaacgcacc acactggaca gccctttggg gaagctggag 60

ctgtctggtt gtgagcaggg tctgcacgaa ataaagctcc tgggcaaggg gacgtctgca 120

gctgatgccg tggaggtccc agcccccgct gcggttctcg gaggtccgga gcccctgatg 180

cagtgcacag cctggctgaa tgcctatttc caccagcccg aggctatcga agagttcccc 240

gtgccggcac ttcaccatcc cgttttccag caagagtcgt tcaccagaca ggtgttatgg 300

aagctgctga aggttgtgaa attcggagaa gtgatttctt accagcaatt agcagccctg 360

gcaggcaacc ccaaagccgc gcgagcagtg ggaggagcaa tgagaggcaa tcctgtcccc 420

atcctcatcc cgtgccacag agtggtctgc agcagcggag ccgtgggcaa ctactccgga 480

ggactggccg tgaaggaatg gcttctggcc catgaaggcc accggttggg gaagccaggc 540

ttgggaggga gctcaggtct ggcaggggcc tggctcaagg gagcgggagc tacctcgggc 600

tccccgcctg ctggccgaaa ctga 624

<210> 2
<211> 22
<212> DNA
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<223> Substrate oligonucleotide containing O6-Benzylguanine at position 14

<220>
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<223> n is O6-benzylguanine

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gtggtgggca gctnaggcgt gg 22

<210> 3
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Sense primer for cloning AGT into pGEX

<400> 3
cgaaatggat ccatggacaa ggattgtgaa atg 33

<210> 4
<211> 43
<212> DNA
<213> Artificial Sequence

<220>
<223> Antisense primer for cloning AGT into pGEX

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gcctttgaat tccgtctttg tagtcgtttc ggccagcagg cgg 43

<210> 5
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Sense primer for mutating K125A, T127A, R128A

<400> 5
gcaaccccgc agccacggca gcagtgggag g 31

<210> 6
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Antisense primer for mutating K125A, T127A, R128A

<400> 6
cctcccactg ctgccgtggc tgcggggttg c 31

<210> 7
<211> 49
<212> DNA
<213> Artificial Sequence

<220>
<223> Sense primer for cloning into eukaryotic pNUC vector

<400> 7
gatcgagcta gcgctaccgg tcgccaccat ggacaaggat tgtgaaatg 49

<210> 8
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Antisense primer for cloning into eukaryotic pNUC vector

<400> 8
gctagggatc ctacgtttcg gccagcaggc g 31

<210> 9
<211> 35
<212> DNA
<213> Artificial Sequence

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<220>
<223> Sense primer for mutating Cys 62 to Ala

<400> 9
gagcccctga tgcaggctac agcctggctg aatgc 35

<210> 10
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Antisense primer for mutating Cys 62 to Ala

<400> 10
gcattcagcc aggctgtagc ctgcatcagg ggctc 35

<210> 11
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> Sense primer for cloning of AGT mutants into phage-display vector

<400> 11
ctactcgcgg cccagccggc catggcggac tacaaagaca tggacaagga ttgtgaaatg 60

<210> 12
<211> 40
<212> DNA
<213> Artificial Sequence

<220>
<223> Antisense primer for cloning of AGT mutants into phage-display vector

<400> 12
ggaattcggc ccccgaggcc gcgtttcggc cagcaggcgg 40

<210> 13
<211> 42
<212> DNA
<213> Artificial Sequence

<220>
<223> Antisense primer for cloning AGT truncated after 182 into pGEX

<400> 13
gcctttgaat tccgtctttg tagtctccca agcctggctt cc 42

<210> 14
<211> 50
<212> DNA
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<223> n is a, c, g, or t

<220>
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<222> (25)..(26)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (31)..(32)
<223> n is a, c, g, or t

<220>
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<223> n is a, c, g, or t

<400> 14
cccaaagccg cgcgagcagt gnnknnkgca nnknnkggca atcctgtccc 50

<210> 15
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Antisense primer for randomisation of codons 131-135

<400> 15
tgctcgcgcg gctttggggt tgcctg 26

<210> 16
<211> 41
<212> DNA
<213> Artificial Sequence

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<223> Sense primer for randomisation of codons 115-116

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<223> n is a, c, g, or t

<400> 16
ggagaagtga tttcttacnn bnnbttagca gccctggcag g 41

<210> 17
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Antisense primer for randomisation of codons 115-116

<400> 17
gtaagaaatc acttctccga atttcac 27

<210> 18
<211> 41
<212> DNA
<213> Artificial Sequence

<220>
<223> Sense primer for randomisation of codons 150-152

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<223> n is a, c, g, or t

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<223> n is a, c, g, or t

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<223> n is a, c, g, or t

<400> 18
ccgtgccaca gagtggtcnn bnnbnnbgga gccgtgggcg g 41

<210> 19
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Antisense primer for randomisation of codons 150-152

<400> 19
gaccactctg tggcacgg 18

<210> 20
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Sense primer for mutating G131K, G132T, M134L, R135S

<400> 20
gcagccacgg cagcagtgaa gacggcactg agtgg 35

<210> 21
<211> 40
<212> DNA
<213> Artificial Sequence

<220>
<223> Antisense primer for mutating G131K, G132T, M134L, R135S

<400> 21
ggatagggac aggattgcca ctcagtgccg tcttcactgc 40

<210> 22
<211> 41
<212> DNA
<213> Artificial Sequence

<220>
<223> Sense primer for mutating Q115S, Q116H

<400> 22
gtgaaattcg gagaagtgat ttcttactct cacttagcag c 41

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<210> 23
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Antisense primer for mutating Q115S, Q116H

<400> 23
cctgccaggg ctgctaagtg agagtaagaa atcac 35

<210> 24
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Sense primer for mutating C150N, S151I, S152N

<400> 24
cgtgccacag agtggtcaat atcaatggag ccg 33

<210> 25
<211> 32
<212> DNA
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<220>
<223> Antisense primer for mutating C150N, S151I, S152N

<400> 25
cgtaaccgcc cacggctcca ttgatattga cc 32

<210> 26
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<220>
<223> Primer for cloning mutant AGT in pET15b

<400> 26
gtcgcatatg gacaaggatt gtgaaatgaa ac 32

<210> 27
<211> 34
<212> DNA
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<220>
<223> Primer for cloning mutant AGT in pET15b

<400> 27
gattacggga tccttatccc aagcctggct tccc 34

<210> 28
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> Antisense primer for cloning truncated AGT in pAK 100

<400> 28
gcaatggaat tcggcccccg aggccgctcc caagcctggc ttccc 45

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<210> 29
<211> 81
<212> DNA
<213> Artificial Sequence

<220>
<223> Sense primer for introducing 12xHis, SbfI and AscI sites

<400> 29
ttatccatgg cacatcatca tcatcatcat catcatcatc atcatcatcc tgcaggtata 60

ggcgcgccta aaagcttctt a 81

<210> 30
<211> 81
<212> DNA
<213> Artificial Sequence

<220>
<223> Antisense primer for introducing 12xHis, SbfI and AscI sites

<400> 30
taagaagctt ttaggcgcgc ctatacctgc aggatgatga tgatgatgat gatgatgatg 60

atgatgatgt gccatggata a 81

<210> 31
<211> 59
<212> DNA
<213> Artificial Sequence

<220>
<223> Sense primer for cloning mutant AGT in pBAD-HisA

<400> 31
ggcctgcagg tgaaaacctg tacttccagg gtatggacaa ggattgtgaa atgaaacgc 59

<210> 32
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> Antisense primer for cloning mutant AGT in pBAD-HisA

<400> 32
aaaaggcgcg ccggatcctt atcccaagcc tggcttcccc aaccg 45

<210> 33
<211> 47
<212> DNA
<213> Artificial Sequence

<220>
<223> Antisense primer for cloning wt AGT in pBAD-HisA

<400> 33
aacggcgcgc cggatcctta gtttcggcca gcaggcgggg agcccga 47

<210> 34
<211> 49
<212> DNA
<213> Artificial Sequence

<220>

<223> Sense primer for cloning AGTM in pEGFP-Nuc

<400> 34
gatcgagcta gcgctaccgg tcgccaccat ggacaaggat tgtgaaatg 49

<210> 35
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> Antisense primer for cloning AGT G160W in pEGFP-Nuc

<400> 35
ccaggcagat ctgtttcggc cagcaggcgg gg 32

<210> 36
<211> 48
<212> DNA
<213> Artificial Sequence

<220>
<223> Antisense primer for cloning AGTM in pEGFP-Nuc

<400> 36
ccaggcagat cttcccaagc ctggcttccc caaccggtgg ccttcatg 48

<210> 37
<211> 33
<212> DNA
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<220>
<223> Sense primer for cloning beta-Gal in pEGFP-Nuc

<400> 37
catcgtctag attatttttg acaccagacc aac 33

<210> 38
<211> 42
<212> DNA
<213> Artificial Sequence

<220>
<223> Antisense primer for cloning beta-Gal in pEGPF-Nuc

<400> 38
gatcgagatc tgggtccgga atgactaaat ctcattcaga ag 42

<210> 39
<211> 25
<212> DNA
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<220>
<223> Sense primer for mutation G160W

<400> 39
caactactcc tggggactgg ccgtg 25

<210> 40
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Antisense primer for mutation G160W

<400> 40
ccagtcccca ggagtagttg cccac 25

<210> 41
<211> 39
<212> DNA
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<220>
<223> Primer for error prone PCR of pAK100 insert, c at position 1 modified with biotin

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ngatccttag acctgaacgc aggtttcccg actggaaag 39

<210> 42
<211> 39
<212> DNA
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<220>
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<400> 42
gcgtcagggt tacaagttca tggtttacca gcgccaaag 39

<210> 43
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Sense primer for amplification of errorprone-PCR product

<400> 43
cgatccttag acctgaacg 19

<210> 44
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Antisense primer for amplification of errorprone-PCR product

<400> 44
gcgtcagggt tacaagttc 19

<210> 45
<211> 48
<212> DNA
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<220>
<223> Sense primer for saturation mutagenesis AGTM 150-154

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<223> n is a, c, g, or t

<400> 45
cgtgccacag agtggtcnnk nnknnknnkn nkgtgggcgg ttacgagg 48

<210> 46
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<223> n is a, c, g, or t

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<221> misc_feature
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<223> n is a, c, g, or t

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<210> 47
<211> 48
<212> DNA
<213> Artificial Sequence

<220>
<223> Sense primer for saturation mutagenesis AGTM 31-35

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<223> n is a, c, g, or t

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<222> (23)..(24)
<223> n is a, c, g, or t

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<222> (26)..(27)
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gcagggtctg cacgaannkn nknnknnknn kaaggggacg tctgcagc 48

<210> 48
<211> 48
<212> DNA
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<220>
<223> Antisense primer for saturation mutagenesis AGTM 31-35

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<220>
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<223> n is a, c, g, or t

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gctgcagacg tccccttmnn mnnmnnmnnm nnttcgtgca gaccctgc 48